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JOURNAL ARTICLE

A CONTINUOUS FLOW BIOASSAY SYSTEM FOR THE EXPOSURE OF MARINE ORGANISMS TO OIL

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ABSTRACT

A continuous flow-through bioassay system is described for exposing marine test organisms and their various life stages to oil. The apparatus consists of two principal stages—one for administering the water-accommodated fractions of oil (WAF) and the second for administering "whole" oil fractions (WF), including the soluble as well as nonsoluble fractions. The oil-contaminated effluent from the system first passes through a chamber to skim off separated oil and finally through a filter which lowers the oil concentration to below one part per million (1 ppm). Short-term lethal and chronic sublethal bioassays of several months duration were conducted in the system and are summarized briefly herein. Biological effects observed during bioassays have ranged from lethal toxicity at oil concentrations of approximately 10 parts per million (ppm) to sublethal behavioral modifications at approximately 10 parts per billion (ppb).

INTRODUCTION

Careful consideration of the potential environmental impact of petroleum has become necessary due to a demand for the development of additional offshore deposits and an increase in related activities along biologically important inshore waters. To facilitate investigations in this area, a gravity fed, continuous flow seawater system has been developed for exposing marine test organisms to various concentrations and fractions of oil.

In comparison to popular static methodology, continuous flow-through systems offer such advantages as replenishment of dissolved oxygen and nutrients vital to test organisms, renewal of volatile and other unstable components of the toxicant, removal of test organisms' metabolic wastes, and more accurate estimates of absolute toxicity.[3, 9] In general, flow-through systems better approximate conditions normally encountered by organisms in their natural environment than do static systems. Continuous flow systems are especially recommended for evaluating the toxicity of crude and refined oils,[1] since these materials include highly volatile compounds that are not readily replenished when static techniques are employed.

Several authors have stressed the need for standardized bioassay methodology to reduce variability among toxicity data reported from various sources.[3, 11, 9, 1, 4] Standardized techniques are particularly necessary for evaluating the effects of oils, due to their complex nature and their unique behavior in water. However, Lichatowich *et al.*[9] pointed out that a standard method for the continuous flow bioassay of crude and refined products does not exist. They claimed that, consequently, much of the observed variance among documented oil toxicity data is attributable to methodological differences. In the same report, these authors described a flow-through bioassay system used by Battelle Pacific Northwest Laboratories for toxicity studies of oils, but also expressed the need for further development of prototypes before the final acceptance of a standard technique. Although recent improvements have been made on the system operated by Battelle,[12] to the best of our knowledge, no further developments have been published by investigators at other laboratories. The present report describes the continuous flow bioassay system now used by the Environmental Protection Agency at Narragansett, R.I., for evaluating effects of oils.

The apparatus consists of two principal stages—one for administering water accommodated fractions of oils (WAF), and the second for administering whole fractions (WF), including soluble as well as nonsoluble fractions. An integral part of the system includes an effluent treatment process to remove floating and water accommodated oil. The system was developed using No. 2 fuel oil as the toxicant and has been operating on a continuous basis for approximately two years with no major problems. All hydrocarbon concentrations were routinely verified by infrared spectrometry.

RESULTS AND DISCUSSIONS

WAF stage

Figure 1 illustrates the WAF stage. Initially this stage consists of a fibergiass, "horseshoe"-shaped oil/water separation chamber (2.44 m long x 81 cm wide x 30 cm high) in which a constant inflow of oil and unfiltered seawater is rigorously mixed through turbulence and then allowed to separate over a series of baffles. Oil is pumped into the separation chamber by a peristaltic pump at a rate of 1.3 ml/min, with seawater entering at the same point by gravity at the rate of 13 l/min from a large seawater reservoir

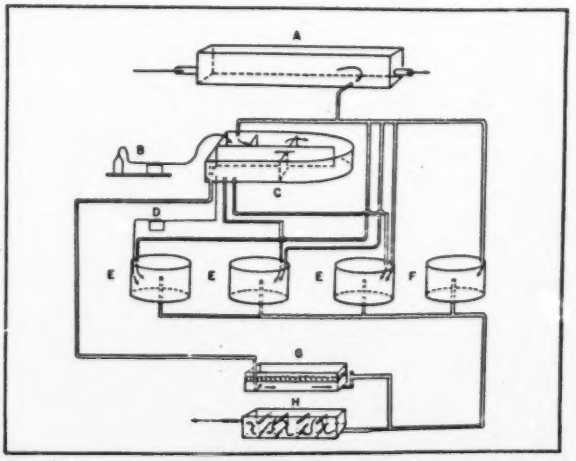

Figure 1. Continuous flow dosing apparatus for water accommodated fractions of oil (WAF): A-seawater reservoir; B-oil supply and peristaltic pump; C-oil separation chamber; D-peristaltic pump for low concentrations; E-experimental dosing tanks; F-control tank; G-waste oil collection chamber; H-filter box with acrylic fibers

located one meter above the system. This combination of flows results in an initial nominal concentration of 100 ppm oil on a volume-to-volume (V/V) basis. Oil and water mix and pass under the first baffle and over the second two baffles. As the mixture continues to flow around the chamber, the nonsoluble fractions float to the surface while the accommodated fractions remain in the water column. Seawater containing WAF is tapped near the end of the separation chamber at mid-depth and is distributed to several 200 l circular, fiberglass dosing tanks. The nonsoluble surface film and excess water flow through a standpipe at the end of the separation chamber and are collected in a smaller (1.10 m long x 50 cm wide x 42 cm high), rectangular, fiberglass waste chamber positioned below.

The concentration of WAF recovered after this mixing and separation process is approximately 15 ppm (V/V) measured analytically. This maximum concentration of WAF is then diluted with clean seawater to reach the desired concentrations of 10, 1, 0.1, and 0.01 ppm within the dosing tanks.

Table 1 reveals the combinations of clean and oiled seawater required to obtain these nominal concentrations, while maintaining a total flow of 3 l/min in each of the dosing tanks. A total flow rate of 3 l/min was proven sufficient for generating adequate supplies of oxygen and for providing enough flow of solution to prevent overloading of test chambers with organisms, which could affect the results of the tests. For example, dissolved oxygen concentrations in test chambers were always at or above 100% saturation. Also, at a flow rate of 3 l/min (4,320 l/day), approximately 8.6 kg of organism biomass per test chamber are required before exceeding a loading of 2 g/liter of solution/day, the upper limit recommended for a variety of test species in flow-through systems.[4]

Since seawater enters the system from a constant head tank, rate of flow into the tanks can be conveniently controlled by the size of orifices cut into the ends of the PVC distribution pipes. Flow rates below 20 ml/min, however, require the use of a peristaltic pump to prevent clogging of necessarily small orifices that are otherwise required.

Table 2 reveals the average concentrations of WAF measured in the tanks during the various monitoring periods. These values were determined following the techniques of Brown *et al.*[3] for total extractable organics. The process first involves the extraction of seawater with 50 ml to 100 ml (for 6 liter water samples) of carbon tetrachloride followed by infrared analysis on a Perkin-Elmer Model 621 Spectrophotometer. The absorbance of the extracted samples is compared to a standard No. 2 fuel oil solution at the absorption maxima of the C-H stretch band determined for the oil used in our studies, i.e., approximately 2930 cm^{-1}. Absorbance units are then converted to parts per million oil in water. The minimum detectable amount using 2 liter samples of seawater with a 40-foid concentration increase in CCl_4 and an absorption path length of 1 cm is approximately 0.01 ppm. The sensitivity is increased to 0.001 ppm when using 6 liter seawater samples with a 60 fold concentration increase, an absorption path length of 5 cm, and 10X ordinate expansion of the signal. This latter more sensitive technique is employed when measuring the low hydrocarbon levels present in the 0.01 ppm tanks. In such cases, concentrations of petroleum hydrocarbons are recorded as the amount in excess of background levels of total organics measured simultaneously in control waters following the same technique.

Table 1. Flow rates required for obtaining nominal concentration of WAF

Nominal concentration (ppm)	Flow rate of oiled seawater	Flow rate of clean seawater (liters/min)
Control	0	3
0.01	2 ml/min[1]	2.998
0.1	20 ml/min	2.98
1.0	200 ml/min	2.8
10.0	2 l/min	1

1. Flow rate actually ranged from 3-12 ml/min

WF stage

Figure 2 illustrates the "whole" fraction (WF) stage. This consists of fiberglass dosing tanks through which mixtures of unpartitioned oil and seawater continuously flow. The mixtures are obtained by passing oil and water through Vigreux columns as they enter each tank. A motor driven skimming device is placed over each tank to keep the medium circulating and to restrict a gross build-up of the nonsoluble fractions on the surface by forcing them down the centrally located standpipe. Seawater flows from the constant head reservoir into each tank at a set rate, while oil is pumped in with the aid of a peristaltic pump (or a syringe pump for slower delivery rates). The flow of oil into each tank was adjusted to yield the desired concentrations of 1.0, 0.1 and 0.01 ppm total petroleum hydrocarbons in the water column. Table 2 reveals actual concentrations measured by IR during the designated monitoring periods. It must be noted that in order to reach desired levels of hydrocarbons in the water column below the surface where test organisms remain, greater amounts on a volume-to-volume basis must be metered into the tanks initially to compensate for the insolubility of certain components. Concentrations of hydrocarbons at the surface, where visible sheens occur, are much greater than the concentrations measured below the surface.

Effluent treatment

The effluent from both stages of the system is treated to reduce to a minimum the amount of oil discharged to the environment. First, the overflow from the separation chamber passes through a waste collection chamber to skim off the nonsoluble surface film. This process removes an average of 78% of the total amount of oil entering the system. The effluent from the waste collection chamber together with the bioassay effluent then passes through a rectangular filter box (1.17 m long x 40 cm wide x 45 cm

Table 2. Analysis of hydrocarbons in test waters

Expected concentration (ppm)	Mean and standard deviation of measured concentration in ppm (V/V) over monitoring period	Water volume extracted (liters)	Monitoring period
Control	<0.01	2	11/74–3/75
	0.016 ± 0.011	6	6/76–10/76[1]
0.01 WAF	0.016 ± 0.012[2]	6	6/76 -10/76[1]
0.1 WAF	0.07 ± 0.03	2	11/74–3/75
	0.08 ± 0.03	2	5/76–7/76[1]
1.0 WAF	0.54 ± 0.17	1	11/74–3/75
	0.82 ± 0.32	1	5/76–7/76[1]
10.0 WAF	10.6 ± 1.3	0.5	11/74–3/76
0.01 WF	0.015 ± 0.003[2]	6	6/76–9/76
0.1 WF	0.17 ± 0.08	2	9/75
1.0 WF	1.42 ± 0.48	1	9/75

1. Courtesy of Brian Melzian, EPA, Narragansett, Rhode Island
2. In excess of total hydrocarbon concentration in control waters

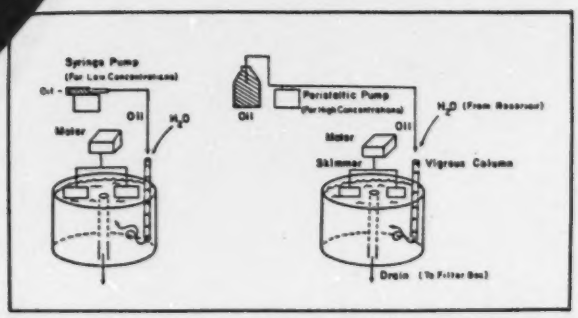

Figure 2. Continuous flow dosing apparatus for whole oil fractions (WF)

high) packed with acrylic fibers to treat the remaining 22%. The filter box is compartmentalized with a series of baffles so that contact with the fiber surfaces is maximized. The fibers apparently remove both dissolved and dispersed oil, resulting in a final effluent concentration below one ppm, provided the fibers are changed approximately three times a week (Moore, *et al.*, 1976). The two treatment processes together remove more than 99% of the oil entering the system, allowing less than 1% (or 25 ml of oil per day) to escape to the environment.

Bioassays

Short-term lethal and chronic sublethal bioassays of several months duration have been conducted in the tanks by several investigators, using embryonic to adult stages of various species of marine fish and macroinvertebrates. For exposing the embryonic, larval and juvenile forms, small glass rearing chambers are used and are positioned outside and adjacent to the larger dosing tanks to receive a continuous flow of test water. Lack of unusual mortalities among control organisms during the various bioassays suggests that the system was effective in providing adequate living conditions for the test organisms.

Results of the bioassays are summarized in Table 3 to illustrate the types of organisms and responses that have been investigated using the system. Effects range from immediate lethal toxicity at approximately 10 ppm to sublethal behavioral modifications, such as reduction in feeding responses, at approximately 10 ppb. Formal documentation of these data will appear in subsequent reports.

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Table 3. Summary of bioassays conducted in the continuous flow system

Species	Reference	Petroleum product	Concentration: nominal and measured	Biological response
Eggs and larvae of minnow (*Menidia menidia*); eggs and juveniles of winter flounder (*Pseudopleuronectes americanus*)	Everich, unpublished data	No. 2 Fuel Oil, WAF	10 ppm (5 ppm by IR)	> 50% mortality of *Menidia* eggs within 2 wks and larvae within 2 days
			1 ppm (0.5–1 ppm by IR	> 50% mortality of flounder eggs within 3 wks at 5°C; 5% mortality within 2 wks at 7–9°C; reduced growth of juvenile flounder within 5 wks at 0.5 ppm
Adult grass shrimp (*Palaemonetes pugio*) and bay scallop (*Argopecten irradians irradians*), juvenile quahog (*Mercenaria mercenaria*)	Hyland, *et al.*, unpublished data	No. 2 Fuel Oil, WAF	10 ppm (10.6 ppm by IR), 1 ppm (0.54 ppm by IR) and 0.1 ppm (0.067 ppm by IR)	At 10.6 ppm, 100% mortality of shrimp within 6 days, scallop within 7 days, quahog within 20 days; 0.54 ppm caused 50% mortality of shrimp within 80 days, and 50% mortality of scallop within 70 days; apparent reduction in filtering activity of clams and scallops at 0.54 and 0.067 ppm; reduction in perception of food and general stamina of shrimp at 0.54 ppm; samples are being processed for hydrocarbon incorporation in tissues and possible histological effects
Adult mussel (*Mytilus edulis*)	Gonzalez, *et al.*, 1976	No. 2 Fuel Oil, WAF	10 ppb (19 ppb by IR)–1 ppm (0.82 ppm by IR)	Significant decrease in filter feeding activity at all concentrations tested; inhibition of byssal thread attachment at 0.82 ppm
Adult mud snail (*Nassarius obsoletus*)	Hyland and Miller 1976	No. 2 Fuel Oil, WAF and WF	10 ppb WF (15 ppb by IR)–1 ppm WF (1.42 ppm by IR); 1 ppm WAF (0.43 ppm by IR)	Significant reduction in chemosensory perception of food at all concentrations

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